JOINT MPH PROGRAM

UNIVERSITY OF GONDAR AND ADDIS CONTINENTAL INSTITUTE OF PUBLIC HEALTH

INCIDENCE AND RISK FACTORS FOR TUBERCULOSIS AMONG PATIENTS RECEIVING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN ADDIS ABABA, ETHIOPIA

By: Yenew Kebede (MD, MSc)

Advisor: Alemayehu Worku (PhD)

A Thesis Submitted to the School of Public Health, University of Gondar in Partial Fulfillment of the Requirements for Degree of Master's in Public Health

June, 2011

ACKNOWLEDGEMETNS

I would like to express my special appreciation to Addis Continental Institute of Public Health and University of Gondar and the staff for their kind support and the opportunity given to pursue the study. I also would like to thank my advisor, Dr.Alemayehu Worku for his valuable and unreserved guidance throughout the study period.

I am indebted to all the staff at Zewditu Memorial Hospital ART Clinic specially Dr. Aster Shewaamare, Sr. Tizita Woldeyesus and Ato Bisrat Tamiru for their genuine dedication and effort demonstrated during data collection.

My acknowledgement also goes to my class mates at Addis Continental Institute of Public Health specially Alemayehu Nigatu for continuous encouragement and support. I would also like to thank Dr. Sisay Alemayehu for the support provided during data analysis and Dr.Tekeste Kebede for his guidance and facilitation with Zewditu Memorial Hospital ART clinic staff.

I appreciate my organization, CDC-Ethiopia Country Office and my colleagues for giving me the time and resources during my study.

Finally, I would like to take this opportunity to thank my beloved family specially my wife, Simegnish Sifir without whose commitment and support I could not have accomplished this work.

TABLE OF CONTENTS

ACRONYMS

AFB	Acid Fast Bacilli
AHR	Adjusted Hazard Ratio
AIDS	Acquired Immuno-Deficiency Syndrome
ART	Antiretroviral Therapy
ARVs	Antiretroviral drugs
CD	Cluster of Differentiation
CDC	Center for Disease Control and Prevention
CI	Confidence Interval
CPT	Cotrimoxazole Preventive Therapy
CRR	Crude rate ratio
D4T	Stavudine
DOTS	Directly Observed Treatment Short course
EFV	Efavirenz
HAART	Highly Active Antiretroviral Therapy
Hgb	Hemoglobin
HIV:	Human Immunodeficiency Virus
IDR	Incidence Density Rate
INH	Isoniazid
IPT	Isoniazid Preventive Therapy
IQR	Inter Quartile Range

IRB Institutional Review Board

IRIS Immune Reconstitution Inflammatory Syndrome

LTBI Latent Tuberculosis Infection

MDR Multi-Drug Resistant

MTB Mycobacterium Tuberculosis

NVP Nevirapine

PEPFAR Presidents Emergency Plan for AIDS Relief

PY Person-Years

3TC Lamivudine

TB Tuberculosis

VCT Voluntary Counseling and Testing

WHO World Health Organization

XDR Extensively Drug Resistant

ZDV Zidovudine

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Background: The protective effect of Highly Active Antiretroviral Therapy (HAART) against Tuberculosis (TB) may be affected by multiple factors. There is little or no information on the long-term impact of HAART on the incidence of TB and associated risk factors in Ethiopia.

Objectives: To assess the incidence, risk factors and treatment outcome of TB in patients on HAART

Methods: This was long term cohort analysis with abstraction of data from medical records of patients at Zewditu Memorial Hospital between September 11, 2006 and October 10, 2010. Patients classified as free of TB at initiation of HAART were included in the analysis. They were routinely monitored and screened for the occurrence of TB. Incidence of TB was estimated and bivariate and multivariate Cox proportional regression model was fitted to determine the risk of tuberculosis.

Results: Total of 546 patients on HAART were enrolled in the analyses. The median duration of follow-up was 23 months [inter quartile range (IQR); 5.8-38.2) and the median baseline CD4 count was 138 cells/µl (IQR; 73-190). The incidence of TB for the cohort was 4.9/100 person-years (95% CI; 3.6-6.2). The incidence significantly declined with duration on HAART reaching 0.2/100 person-years in the fourth year (P<0.001 for trend). More than 60% of cases occurred in the first three months. Baseline CD4 cell count <100 cells/µl [adjusted hazard ratio (AHR); 2.8 (95% CI; 1.6-4.9, P<0.001) and WHO clinical stage 3 or 4 (AHR; 2.2 (95%CI; 1.1-4.4, P=0.03) were independent risk factors for the occurrence of TB during HAART. The highest incidence of TB was observed among patients with CD4 cell count <50 cells/µL (10.2 cases /100PY (95% CI; 4.8-15.5). Isoniazid preventive therapy (IPT) was not significantly associated with an increased TB incidence in multivariate analysis but patients who received IPT have 10% higher TB-free survival proportion compared with those with no IPT (P=0.03, log rank test). TB during HAART carried bad prognosis with death rate of 29.1/100 person-years compared with 2.6/100 person-years death rate for non-TB cases (P=0.02).

Conclusion: Incidence of TB is highest in the first 3 months and continues to decline in the first four years. Advanced pre-treatment immunodeficiency is an important predictor of occurrence of TB during HAART. Isoniazid preventive therapy may further reduce incidence of TB in patients receiving HAART.

Recommendation: Comprehensive TB control measures including stringent screening of TB before initiation of HAART should be implemented consistently to reduce burden of TB among HIV infected individuals. HAART should be initiated at higher CD4 cell count as recommended in the national guideline to reduce incidence of TB. Other adjunct strategies like IPT if implemented consistently can help to further reduce risk of TB in HIV patients.

1. INTRODUCTION

The interaction between Tuberculosis (TB) and Human Immunodeficiency Virus (HIV) infection is complex. HIV infection weakens the immune system and increases susceptibility of individuals to TB. HIV infection is also an established risk factor for reactivation, re-infection and progression of latent TB infection to active diseases. Moreover, it alters the clinical presentation of TB, complicates the follow-up and compromises the response to anti-TB treatment (1). HIV-positive people are 20 times at higher risk to develop TB compared to HIV-negative people in countries with a generalized HIV epidemic (2). Therefore, TB is the most common opportunistic infection among HIV-infected patients, who remain at higher risk for TB throughout the course of their disease specially in African setting.

The recent increase in worldwide prevalence of HIV infection has contributed to the rising global incidence of tuberculosis. Of the estimated 9.4 million new TB cases that occurred worldwide in 2009 for example, 1.2 million (13%) were associated with HIV and the African region accounted for 80% of the global burden of HIV-associated TB. In Ethiopia, infection with TB is a major public health problem with an estimated annual incidence of 168 cases per 100,000 population for smear positive tuberculosis and 378 cases per 100,000 populations for all forms of TB (2). Similarly, the rate of TB/HIV co-infection is high and depends on the prevalence of HIV infection in community. Studies have shown that the TB/HIV co-infection rate in Ethiopia was variable ranging from 18-52% (3-6). In addition, one population-based study demonstrated strong positive association between the prevalence of HIV infection and incidence of all forms of TB (7).

The current TB control strategy of case-finding and directly observed treatment of sputum smear-positive patients using short-course anti-tuberculosis treatment (DOTS) has not been effective to contain HIV-related TB epidemic (8). The World Health Organization (WHO) formulated a strategic framework where highly active antiretroviral treatment (HAART) is an important element (8). Studies have shown that TB incidence is reduced by two-thirds in HIV infected cohorts receiving HAART in both high-income and resource-limited settings (9, 10). The effect of HAART in reducing TB incidence has been shown to be time-dependant with most benefits occurring during the first 2-3 years of treatment. The risk reduction is directly related to increases in blood CD4 cell counts and to the restoration of TB-specific immune responses. CD4 cell count increase from <100 cells/µl to >500 cells/ µl was associated with a 10-fold reduction of risk of TB (11). In Ethiopia almost 90% reduction in TB incidence was observed during one year follow-up period in HIV positive cohorts who received HAART compared to Pre-antiretroviral therapy (ART) patients followed in the same facility (12).

Increasing evidences suggest that, unlike low virulent pathogens, significant rates of TB persist during HAART (13, 14). Even though the incidence of TB is greatly diminished during treatment, the risk remains substantially higher. This has important implications for the extent to which HAART may assist in TB control in low income countries (14). Studies have identified factors that are associated with high incidence of TB during HAART. Low baseline CD4 count (<100cells/µl), WHO clinical stage of the disease (Stage 3 or 4), younger age and previous history of TB were important risk factors for high incidence of TB among patients receiving HAART (15, 16). The use of Isoniazid preventive therapy (IPT) was associated with lower risks of TB (16). Another study has described past history of TB as the only factor for an increased

risk of TB during HAART (17). Most studies used data from cohorts with short median duration of follow-up. Long term effect of HAART on TB incidence needs to be studied further.

In Ethiopia, much has not been done to know long term effect of HAART on the incidence of TB. One study in a district hospital in Southern region showed 3.7 per 100 person years (PY) of observation TB incidence in patients receiving HAART (12). This was based on the emergence of 6 new TB cases only after 50 weeks of follow-up of 180 patients receiving HAART. Risk factors for an increased incidence of TB during HAART have not been well characterized. Understanding the different factors related to the occurrence of TB during ART is critical for better care of patients and will also be helpful to guide the programs. This study was therefore, aimed at determination of the incidence of TB and associated risk factors in patients receiving HAART at Zewditu Memorial Hospital from September 11, 2006 to October 10, 2010.

2. LITERATURE REVIEW

2.1. Human Immunodeficiency Virus (HIV) infection and global burden of Tuberculosis

Infection with *Mycobacterium tuberculosis* (MTB) remains one of the leading causes of morbidity and mortality globally (18). The growing burden of multidrug-resistant (MDR) and extensively drug-resistant (XDR) cases of TB poses additional challenges to TB case management. Of the estimated 9.4 million new cases of TB that occurred in 2009, 1.2 million (13%) were co-infected with HIV. African region accounted for approximately 80% of the burden of TB-HIV co-infections, followed by South-East Asia (11%). In 2009, there were 380,000 TB-related deaths among HIV-positive patients accounting for 23% of the global HIV/AIDS mortality. Globally, an estimated 33 million people are infected with HIV (2).

HIV-positive people are about 20 times more likely than HIV-negative people to develop TB in countries with a generalized HIV epidemic (2). Ethiopia is home for an estimated 1.1 million people living with HIV with adult HIV prevalence estimated to be between 1.4-2.8% (19). Infection with TB is also a major public health problem in Ethiopia with an estimated annual incidence of 168 cases per 100,000 population for smear positive tuberculosis and 378 cases per 100,000 populations for all forms of TB (2). HIV related tuberculosis is an important challenge for the TB control program. The TB/HIV co-infection rate is variable (18-57%) depending on the prevalence of HIV in the community (3-6).

2.2. HIV infection and DOTs program

The current TB control strategy-Directly Observed Treatment Short course (DOTS) was not effective in curving the TB epidemic specially in countries with high burden of both diseases. This relates to the fundamental epidemiological interaction between TB and HIV both of which are important public health problems in African settings. The principle underlying DOTS is to diagnose and effectively treat infectious pulmonary TB cases and thereby reduce onward transmission and avert secondary cases. However, DOTS does not prevent reactivation of latent TB infection (LTBI) which commonly is the case with HIV co-infection. In those with HIV co-infection, subsequent risk of developing TB through reactivation of LTBI is extremely high, with overall rates reaching as high as 20-30% per year specially in those with the most advanced immunodeficiency (20, 21). In addition, DOTS does not reduce the very high susceptibility of HIV-infected individuals to develop rapidly progressive disease following exposure.

The reduction in the transmission risk in the community achieved through expansion of DOTS program was out-weighed by many-folds by the greatly increased risk of rapidly progressive disease in HIV-infected people who are nevertheless exposed. Major increases in incidence rates of TB may further contribute to transmission, although this is off-set to some extent by the fact that HIV-associated TB cases are generally less infectious than disease in HIV-uninfected people (21). Without addressing the impact of HIV on TB epidemic, DOTS and other interventions will not be successful in curving the tide of TB epidemic. High incidence rates of HIV-associated TB and associated high mortality risk are driven by immunodeficiency as reflected by the blood CD4 cell count. Without addressing this, approaches to TB control are flawed and hence World Health Organization (WHO) launched a Stop TB strategy in 2006 which emphasizes the TB/HIV co-

infection (22). Therefore, HAART must clearly be central to both TB treatment and prevention strategies.

2.3. Effect of HAART on TB incidence

Studies have shown that TB incidence is reduced by two-thirds in HIV infected cohorts receiving HAART in both high-income and resource-limited settings (9, 10). One prospective cohort study in Ethiopia indicated that the incidence of TB was 3.7/100 person-years (PY) in patients receiving HAART and 11.1/100 PY in Pre-ART patients indicating a 3 times lower risk of TB among those taking HAART (12). This was however; based on 185 pre-ART and 180 ART patients followed for less than a year and the long term effect of HAART on TB was not well described. The effect of HAART in reducing TB incidence has been shown to be time-dependant with most benefits occurring during the first 2-3 years of treatment (10). The risk reduction is directly related to increases in blood CD4 cell counts and to the restoration of TB-specific immune responses. CD4 cell count increase from <100 cells/µl to >500 cells/ µl was associated with a 10-fold reduction of risk of TB (11). The TB preventive effects of HAART are observed across a wide spectrum of baseline CD4 cell counts, including those with the very lowest counts and benefits work well in those with and without evidence of latent tuberculosis infections. HAART also reduces TB risk in those with a history of previous TB, with an approximate halving of TB recurrence rates. Thus, HAART is potentially a potent preventive tool for addressing the HIV-associated TB epidemic. In addition, HAART transforms the prognosis of patients with HIV-associated TB, reducing mortality risk by 64-95% (10). It is therefore, absolutely essential that all patients with TB are HIV-tested so that those who test positive may receive the benefits of HAART, Isoniazid prophylactic therapy (IPT) and co-trimoxazole

preventive therapy (CPT). The national guideline in Ethiopia recommends HIV testing for every TB patients and TB screening for every HIV patients.

2.4. Occurrence of TB during HAART

Even though the TB risk reduction observed in individuals receiving HAART is very substantial, the long term effect of HAART in reducing the risk of TB may be limited due to multiple factors. Limited proportion of HIV-infected population has been reached and achieving high coverage will be resource- intensive and challenging. For example, in Ethiopia less than 50% of HIV positive patients that required treatment are currently receiving therapy. Patients entering ART program typically have low CD4 cell counts and many of them could present with TB as a first symptom. More than 30% of HIV positive patients in Ethiopia come with a CD4 cell count less than 100 cells/µl (23). Among HIV-infected individuals who are not receiving HAART, risk of TB increases with increasing HIV-associated immunodeficiency (24). Patients with most advanced pretreatment immunodeficiency as indicated by both blood CD4 cell count and WHO clinical stage retain the highest risk of TB during HAART. This may reflect the fact that the greater the degree of pre-HAART immunodeficiency, the more prolonged the period of treatment required to restore immune function. Moreover, advanced pretreatment immunodeficiency also limits the extent to which immune functions can be restored in the long term (25, 26). The incidence of TB for example, was 3.6 times higher among patients whose CD4 count during the initiation of ART was <100 cells/µl compared to those whose CD4 cell count was $\geq$100 cells/µl (15). Treatment guideline in Ethiopia considers advanced disease (WHO stage 3 or 4) and CD4 cell counts < 200 cells/µl for initiation of HAART (27). Treatment according to these recommendations may limit the extent to which long-term TB-specific

immune responses may be restored in many patients and this may restrict the potential benefits of widespread use of HAART in a TB control program to reduce the incidence of tuberculosis.

During long-term HAART, TB incidence rates remain several-fold higher than rates among HIV-uninfected people living in the same community (9, 10) and this risk is likely to be sustained throughout their life-span. Their life-span in turn is greatly increased by HAART and so cumulative risk of TB over time will remain high. TB risk may remain high in some patients due to poor immunological response to HAART, poor treatment adherence, ART failure, nosocomial TB transmission (resulting from treatment in hospital) or being lost to follow-up. As a result of the above factors, the cumulative lifetime risk of TB in patients enrolled in ART programs may therefore remain extremely high (9-11). Tuberculosis recurrence after previous diagnosis and treatment results either from endogenous relapse or disease after re-infection with a different mycobacterium strains (28). Recurrence is significantly increased in HIV-infected persons. Therefore, prior history of tuberculosis is an important risk factor for subsequent occurrence of TB in patients receiving HAART. The incidence in patients with a prior history of tuberculosis can be as high as 11.3/100 person-years compared with 3.0/100 person-years in those without prior tuberculosis with adjusted hazard ratio of 4.6 (17). Both re-infection and relapse play a part, with re-infection being more likely in HIV-infected than uninfected persons, especially in high transmission areas, as well as in persons in whom recurrence is more distant in time from initial treatment (29, 30).

The use of IPT reduces the risk of TB but combination of IPT and HAART lowers the risk significantly suggesting that the provision of antiretroviral drugs (ARVs) and tuberculosis

preventive therapy could have a more substantial impact on HIV-related tuberculosis than either strategy alone (16). In terms of timing, the incidence of TB was very high in the first three months after HAART initiation, especially in patients with very low CD4 counts, and likely contributes to high early mortality (31).

2.5. Socio-demographic Factors and Incidence of TB among patients receiving HAART

Younger age was identified as risk factor for TB during HAART. The mechanism was unclear but was suggested possibly to reflect behavioral differences that affect exposure to HIV infection (15, 16). Male gender was also associated with the higher risk of TB in a multivariate analysis even though sound scientific explanation was not given (31). Other socio-demographic factors were not significantly associated with an increased risk of TB during HAART.

2.6. Rationale of the study

In Ethiopia, there has not been much work to describe the long term effect of HAART on the incidence of TB. Risk factors for TB among HIV positive patients receiving HAART were not well characterized. Such information helps in the management of patients and provides guidance to the programs. In addition, it will also serve as baseline information for further impact analysis of effectiveness of HAART for the reduction of the TB burden. Therefore, this study was aimed at determination of the incidence and risk factors of TB in patients receiving HAART at Zewditu Memorial hospital from September 11, 2006 to October 10, 2010. The hospital is model ART and VCT center with more than 6000 patients currently on HAART.

3. OBJECTIVES

3.1. General Objective

- To assess the incidence, risk factors and treatment outcome for TB in patients receiving HAART

3.2. Specific Objectives

- To determine the incidence of TB in patients receiving HAART
- To describe risk factors of TB among patients receiving HAART
- To explore treatment outcome of incident TB cases receiving HAART

4. METHODS

4.1 Study area/Context

ART clinic at Zewditu Memorial Hospital was established in July 2003. The free ART program in this clinic was initiated in March 2005. This is the first and the largest clinic in the country and serving about 15,335 clients. Each day, on average, 20 new clients and 200 repeat cases are served. It is now an important ART training and research center and continues to be model ART and Voluntary Counseling and Testing (VCT) center in Ethiopia. The main activities of the ART clinic are enrolling HIV positive patients in to chronic care and follow-up, assessment of patients for ART eligibility, adherence counseling and initiation of ART, and prophylaxis and treatment of opportunistic infections. The TB/HIV collaborative activities like intensive case finding, INH prophylaxis, infection prevention and referral of TB patients to DOTs clinic are core components of routine patient care.

Between July 2003-2010, 5,977 adult and 506 pediatric patients have been taking HAART and 21 patients were on second line treatment. However, the number of patients ever started on HAART was very large, 9881. There were 1, 372 dropouts, 1,687 transferred outs and 826 deaths in the same period as factors for the gap between ever started and currently on HAA RT. Twelve percent of HIV positive patients had TB and 25.4% of TB patients were positive for HIV. The clinic has two medical doctors providing ART services, 5 regular nurses, 3 advanced ART nurses, 5 data clerks, 1 pharmacist, 2 druggists, 3 lab technicians, 1 pediatrics ART Physician, 2 pediatrics ART nurses and 7 adherence supporters (people living with HIV). The hospital uses standardized monitoring and evaluation tools and the data collection and management process is well controlled and supported by electronic data back-up and processing.

4.2. Source and Study Population

The study was conducted at Zewditu Memorial Hospital where there were 5,997 patients receiving HAART. The actual study population was patients newly enrolled in ART program from September 11, 2006 to October 10, 2010. This period was selected since the facility started full implementation of standardized formats, documentation and recording system in regular manner during this period. This was critical since the study was based on secondary data and it was important to make sure that important variables for the study should be available for all enrolled subjects in the study. The likelihood of obtaining complete information on the variables for the study was very high during this period.

4.2.1. Inclusion criteria

- All HIV positive patients with age greater than or equal to 15 years who started HAART between September 11, 2006 to October 10, 2010.
- HIV positive patients on HAART, who have baseline CD4 determination and at least one additional prospective follow-up at the facility

4.2.2. Exclusion criteria

- HIV positive patients on HAART with incomplete clinical records (intake forms, Pre-ART and ART registers and follow-up forms)
- All transferred in HIV patients on HAART. Baseline clinical and socio-demographic information for these patients exists only in facilities where they started HAART

- HIV positive patients who have been taking treatment for tuberculosis at the time of initiation of HAART (however, all patients who have completed tuberculosis treatment before initiation of HAART or have TB treatment history were included)

The risk of TB among patients receiving HAART was assessed using the person-time method. Follow-up was censored at date of TB diagnosis, or death or the date of the most recent visit. Patients who died during the follow-up were censored on the date of their last clinic visit, as it was difficult to verify the exact dates of death. All the exposures and outcomes were abstracted from patient medical records. The main outcome of interest was the occurrence of TB among patients taking HAART at any point in the follow up period. Tuberculosis was diagnosed in patients presenting with the signs and symptoms suggestive of tuberculosis on the basis of chest radiographs, sputum and other body fluid smear microscopy (AFB), fine needle aspiration (FNA) and response to anti-tuberculosis therapy.

4.3. Study Design

This was a retrospective cohort study on adult patients taking HAART and being followed at Zewditu Memorial Hospital from September 11, 2006 to October 10, 2010. Information was retrieved on the socio-demographic factors, WHO stage of HIV at enrollment, CD4 counts at enrollment and on intervals then after (6 month, 12 month, 18 month, 24 month, 30 month and 36 months of follow-up), viral load information if available, previous history of TB, occurrence of TB during follows up and length of follow-up before developing TB. In addition, the type of tuberculosis (smear positive pulmonary, smear negative pulmonary or extra-pulmonary) was noted. Data on IPT and outcome of treatment of patients with TB during the follow up period

were collected. The data on different dependent and independent variables were collected from ART register, HIV care/follow up charts, TB treatment register and medical records.

4.4. Sample Size and Sampling Procedure

Studies have indicated that 3-10% of patients receiving HAART develop any form of tuberculosis in subsequent years (12, 15, 31). For the first objective of the study, by taking 10% as proportion of patients developing new TB while receiving HAART, with a precision of 3% and confidence interval of 95%, the sample size was:

$n=Z\alpha/2^2p(1-p)/d^2$

$n=1.96^2(.1x.9)/.03^2$

n=384

To assess the risk factors for tuberculosis in patients receiving HAART, the difference between two sample proportions sample size calculation formula was employed. Studies have indicated that baseline CD4 count is an important factor associated with the subsequent occurrence of TB in patients receiving HAART. One long term follow-up study in South Africa for example, has indicated that the incidence of TB in patients enrolled in to ART program at baseline CD4 count of less than 100 cells/µl was 5.7/100 person-years (12 new TB cases among 73 patients followed) as compared to 1.6/100 person-years (15 new TB cases from 273 patients followed) for those enrolled at baseline CD4 count of $\geq$100 cells/µl [Adjusted risk ratio 2.38, p=0.04] (15). In Ethiopia, facility reports have indicated that one-third of patients start HAART at CD4 count <100 cells/µl. At 95% confidence interval and 80% power of test and 1:2 ratio between exposed

(CD4 count <100) and unexposed (CD4 count ≥100 cells/µl), the sample size for each categories was:

$$n1 = \frac{Z\alpha/_2\sqrt{[1+1/r]P(1-P)} + Z_\beta\sqrt{p1(1-p1)+p2(1-p2)/r}}{(p1+p2)^2}$$

n1=the required sample size for the exposed (CD4 <100)

n2=the required sample size for the non-exposed (CD4 ≥100)

n1:n2=1:2

p1=proportion of TB in exposed

p2=proportion of TB in non-exposed

P=p1+rp2/1+r

r=n2/n1

α=type-I error (0.05)

$Z_{\alpha/2=}$Critical value at 95% level of confidence

Z_β=standard normal distribution value corresponding to the power

n1= **184** and n2=**362** making the total sample size **546**. Similar calculations were made for other variables but the sample size calculated for CD4 count was maximum number and was taken to be the final sample size for the study.

In cohort study, the point of entry of patients to the study and the duration of follow-up are important parameters. Therefore, 546 subjects (184 with CD4 count <100cells/µl and 362 with baseline CD4 count ≥100cells/µl) were distributed to each month of the study period from September 2006 to October 2010 proportional to the number of subjects in each categories

enrolled in each month. Once this was determined, the actual study subjects from each month were selected by simple random sampling. Identification number of subjects enrolled to HAART in each month for both categories was retrieved from the electronic data base and the study subjects were selected by the lottery method until the sample size allocated for that month was reached and similar procedures were applied to the entire study period. The occurrence of new tuberculosis (either re-infection or reactivation) is the dependent variable and all socio-demographic and clinical characteristics of patients are independent variables.

4.5. Data Collection Procedure

Information on socio-demographic variables, baseline and subsequent CD4 test results, whether patients were on first or second line ART, the previous TB treatment status, occurrence of TB during follow-up, treatment outcome and other related variables were collected from the patient records (ART register, HIV care/follow up charts, TB treatment register and medical records) using well designed and pre-tested questionnaire. The available information on the patient records were first observed and appropriate data collection tool was developed with an input by experts working at the site.

Zewditu memorial hospital is one of the hospitals where health management information system (HMIS) is implemented. HMIS is a very important initiative by Federal Ministry of Health (FMOH) and hence there is stringent data quality control system in place. The data management system gets a lot of support from partners. President's Emergency Plan for AIDS relief (PEPFAR)-Ethiopia and John Hopkins University (JHU), partner of United States Centers for Disease Control and Prevention in Ethiopia (CDC-Ethiopia), has been providing technical

support throughout data collection process. The data base is in the electronic system and this facilitated retrieval of patient registration number. Patient charts were retrieved using the patient registration number which was a unique identifier. All data were retrieved from the patient follow-up charts. For missing elements like treatment outcome and INH provision especially for incident TB cases, pre-ART and TB registers were additionally used. Quality of data has been checked and there were dedicated data clerks working in ART section. The JHU monitoring and evaluation team and clinical team at Zewditu Memorial Hospital regularly monitor the quality of the data.

One trained nurse working at TB clinic and one data manager in the ART section were employed for the data collection. Even though both know all the processes and procedures of data management in the facility, they were trained on how to retrieve data for this study and were provided with written standard operating procedures. They were briefed on the definition of variables on the questionnaire and how to retrieve the variables from their data base system. The sample retrieval process was closely monitored by the principal investigator throughout the data collection period. Completed questionnaires were checked regularly for completeness of information and any gaps identified were immediately communicated to the data collection team.

4.6. Data Analysis

Data was entered to Epi Info version 3.3.2 and was cleaned before analysis. SPSS software (PASW statistics 18, 1993-2007) was used for the analysis. Subjects in each CD4 count category were characterized by their socio-demographic and clinical characteristics. The difference in proportions was compared by X^2 test. The incidence density rate (IDR) was calculated for the

entire study period for the two categories of study subjects. IDR was also calculated for each year of the study to see if there was any difference in the IDR through time. IDR was defined as the number of new episodes of TB occurring per 100 patient-years of observation. This analysis was further stratified by WHO clinical staging (stage 3 or 4 versus stage 1or 2), socioeconomic status, previous history of TB, age, base-line hemoglobin (<10 gm/dl or ≥10 gm/dl) and gender. Subjects were categorized by the CD4 count at the design stage. The CD4 count <100 was used as cutoff as this has been indicated as significantly and independently associated with risk of TB in another cohort study elsewhere (15). However, incidence was also calculated for low CD4 cell count (<50 cells/µl). Kaplan-Meier plots were used for analysis of probabilities of not developing TB. TB-free survival was defined as the time from enrolment to HAART to the date of TB diagnosis, death from any cause or the last follow-up visit. TB-free survival again was stratified further by baseline immunological and clinical status and was compared using the generalized log rank test. Bivariate and multivariate Cox proportional hazards regression models were fitted to determine the risk of TB, which was expressed as a rate ratio. Variables were considered for inclusion into the multivariate model if their p-value in bivariate analysis was ≤0.3. All tests were two sided and a P value of 0.05 was considered significant.

4.7. Operational Definitions

- New or incident TB in this study was defined as the occurrence of TB (new infection or reactivation of latent TB) in HIV patients any time after HAART. The type of TB can be smear positive pulmonary, smear negative pulmonary, extra-pulmonary or disseminated tuberculosis as identified by signs and symptoms, laboratory and/or X-ray diagnosis.

- Duration of follow-up was the time from initiation of ART until occurrence of TB for the incident TB cases and until the last follow-up period for non-TB subjects, transferred outs, dead or lost to follow-up.

4.8. Ethical Consideration

This study was based on the secondary data collected after routine clinical care for HIV patients. Patients get the service according to the national guidelines and data were recorded after each service. Patients have been followed based on program requirements. There was no any intention to collect any additional information from patients and there was no collection of any patient samples for this purpose. In addition, the study protocol was cleared by the Institutional Review Board (IRB) of University of Gondar. Support letter was obtained from Addis Ababa Regional Health Bureau. Permission was sought from the management of the Zewditu Memorial hospital to use the secondary data for the purpose of this study. The results were kept confidential and there were no any personal identifiers attached during data retrieval in this study. The information generated from this study will be helpful for better management of patients with TB/HIV co-infection and will be an input for guiding the program. It can also serve as baseline information for long term impact analysis of HAART on prevention and control of tuberculosis.

5. RESULTS

5.1. Cohort Characteristics

Between September 11, 2006 and October 10, 2010, cohorts of 546 patients receiving HAART at Zewditu Memorial Hospital, Addis Ababa were retrospectively selected. Data were collected through a retrospective medical record review. At base line, the median age of the cohort was 36 years (range, 17-70) with most study subjects under the age group of 30-44 years. Females accounted for 58.8% of patients enrolled and 84.5% of study subjects had secondary education or lower. Majority (71.4%) of subjects was either married or single and 80.4% were orthodox Christians. Significant number (44.9%) was unemployed. The median base-line CD4 cell count was138 cells/µl (IQR; 73-190, range; 5-638). Total of 184 (33.7%) of patients had baseline CD4 count of <100 cells/ µl and 362 (66.3%) had baseline CD4 cell count of ≥100 cells/ µl. Forty-eight (8.8%) of subjects had baseline hemoglobin (Hgb) of <10gm/dl, 355 (65%) of patients had symptomatic disease (WHO clinical stages 3 or 4); and 94 (17.2%) had a previous episode of tuberculosis (Table 1).

Table 1: Baseline characteristics of patients on HAART at Zewditu Memorial Hospital September 11, 2006 to October 10, 2010

Characteristics	CD4 <100 cells/µl Frequency (%) n=184	CD4 ≥ 100 cells/µl Frequency (%) n=362	Total Frequency (%) N=546
Age			
15-29	41 (22.3)	65 (17.9)	106 (19.4)
30-44	115 (62.5)	233 (64.4)	348 (63.7)
≥45	28 (15.2)	64 (17.7)	92 (16.8)
Sex			
Male	84 (45.7)	141 (39.0)	225 (41.2)
Female	100 (54.3)	221 (61.0)	321 (58.8)
Marital Status			
Never married	60 (32.6)	83 (22.9)	143 (26.2)
Married	69 (37.5)	178 (49.2)	247 (45.2)
Separated/divorced	26 (14.1)	45 (12.4)	71 (13.0)
Widowed	29 (15.8)	56 (15.5)	85 (15.6)
Religion			
Orthodox	142 (77.2)	297 (82.0)	439 (80.4)
Muslim	19 (10.3)	24 (6.7)	43 (7.9)
Protestant	22 (12.0)	38 (10.5)	60 (11.0)
Catholic	1 (0.5)	-	1 (0.2)
Others	-	3 (0.8)	3 (0.5)
Educational status			
No education	26 (14.1)	36 (9.9)	62 (11.4)
Primary	53 (28.8)	124 (34.2)	177 (32.4)
Secondary	79 (42.9)	143 (39.5)	222 (40.7)
Tertiary	25 (13.7)	53 (14.7)	78 (14.3)
NA	1 (0.5)	6 (1.7)	7 (1.3)
Occupation			
Employed	64 (34.8)	125 (34.5)	189 (34.6)
Unemployed	85 (46.2)	160 (44.2)	245 (44.9)
NA	35 (19.0)	77 (21.3)	112 (20.5)
WHO stage			
Stage 1 or 2	44 (23.9)	147 (40.6)	191 (35)
Stage 3 or 4	140 (76.1)	215 (59.4)	355 (65)
Baseline hemoglobin			
<10gm/dl	23 (12.5)	25 (6.9)	48 (8.8)
≥10gm/dl	161 (87.5)	334 (92.3)	495 (90.7)
NA	-	3 (0.8)	3 (0.5)
Previous TB history			
Yes	43 (23.4)	51 (14.1)	94 (17.2)
NO	140 (76.1)	305 (84.2)	445 (81.5)
NA	1 (0.5)	6 (1.7)	7 (1.3)

TB=tuberculosis, NA=not available, WHO=World Health Organization

The median duration of follow-up was 23 months (IQR; 5.8-38.2, range; 0.3-52.6). At the end of follow-up, 487 (89.2%) of patients were still on treatment, 26 (4.8%) were lost to follow-up or transferred out and 33 (6%) had died. The predominant highly active antiretroviral (HAART) regimens initially prescribed were combination of Zidovudine, Lamivudine and nevirapine (30.4%), zidovudine, lamivudine and efivarenz (24.7%) followed by stavudine, lamivudine and nevirapine (17.6%). Only 3 (0.5%) patients were switched to second line anti-retroviral therapy due to treatment failure and 144 (26.4%) of subjects developed side effects to medication that required regiment substitution. Cotrimoxazole preventive therapy (CPT) was provided to 511 (93.6%) of patients and 65 (11.9%) received IPT (Table 2).

Table 2: Characteristics after follow-up of patients on HAART at Zewditu Memorial Hospital September 11, 2006 to October 10, 2010

Characteristics	CD4 <100 cells/µl Frequency (%) n=184	CD4 ≥ 100 cells/µl Frequency (%) n=362	Total Frequency (%) N=546
Cohort Status (last follow-up date)			
Still on HAART	148 (80.4)	339 (93.7)	487 (89.2)
Lost to follow-up or transferred out	11 (6.0)	15 (4.1)	26 (4.8)
Dead	25 (13.6)	8 (2.2)	33 (6.0)
Initial regimen			
D4T/3TC/NVP	40 (21.7)	56 (15.5)	96 (17.6)
ZDV/3TC/NVP	49 (26.6)	117 (32.3)	166 (30.4)
D4T/3TC/EFV	35 (19.0)	29 (8.0)	64 (11.7)
ZDV/3TC/EFV	43 (23.5)	92 (25.4)	135 (24.70
Others	17 (9.2)	68 (18.8)	85 (15.6)
Regimen switched to Second line			
Yes	-	3 (0.8)	3 (0.5)
No	184 (100)	359 (99.2)	543 (99.5)
Developed side effect for HAART			
Yes	45 (24.5)	83 (22.9)	128 (23.4)
No	139 (75.5)	279 (77.1)	418 (76.6)
IPT provided			
Yes	3 (1.6)	62 (17.1)	65 (11.9)
No	181 (98.4)	300 (82.9)	481 (88.1)
CPT provided			
Yes	172 (93.5)	339 (93.7)	511 (93.6)
No	12 (6.5)	23 (6.3)	35 (6.4)

IPT= Isoniazid preventive therapy; CPT=cotrimoxazole preventive therapy

5.2. New Tuberculosis Cases

There were 51 new TB cases out of the 546 patients included in the analysis (9.3%). Among the cases, 27 (53%) were extra-pulmonary and/or disseminated, 22 (43%) were smear negative pulmonary and 2 (4%) were smear positive pulmonary TB cases. The median age for the TB cases was 35 years with range of 24-53 and females accounted for 54.9% of new TB cases. Six (11.8%) of cases had past TB history, only one case had received IPT while 96.1% of new TB

cases had received CPT. Eighteen cases (35.3%) developed side effects for anti-retroviral treatment that warranted regimen substitution (Table 3). The median duration of follow-up for the TB cases was 1.8 months (IQR; 0.7-7.0, range; 0.3-43.7)). The median base-line CD4 cell count was 86 cells/ µl (IQR 45-139, range; 7-247). Twenty-four cases (47.1%) were on NVP containing regimen, which in 39% of cases required substitution by EFV due to fear of liver toxicity. The cases are presumptive cases based on sputum smear microscopy, chest x- ray, pathological evidence, clinical signs and symptoms and response to therapy. TB culture was not being done routinely. All 51 episodes of TB occurred with the first four years of follow-up and majority (60.8%) occurred in the first 3 months after initiation of HAART and 82.4% of cases had already occurred in the first year of follow-up. Only 3.9% of cases occurred after 2 years on HAART (Fig 1.).

5.3. Tuberculosis treatment outcome for incident cases

Forty of the new tuberculosis cases (78.4%) had completed treatment or were cured, 7 (13.7%) had died and more deaths for TB cases occurred among patients with CD4 count of less than 100 cells/µ1 (85.7%), 2 (3.9%) were still on treatment during the last follow-up but treatment outcome could not be verified for 2 (3.9%) patients (Table 3). Of 33 deaths in the entire cohort, 7 (21.2%) were among those diagnosed with TB during the follow-up even though verification of causes of death was not possible from records. The mortality rate was compared between patients receiving HAART who remained free of TB until the last date of follow-up and those who developed TB in the course of treatment. There were 7 deaths among new TB cases after 24.04 person-years of follow-up giving death rate of 29.1/100 person-years (95% CI; 7.5-50.7). On the other hand, there were 26 deaths among patients on HAART who remained free of TB until the last follow-up period after 1015.32 person-years of follow-up; death rate of 2.6 per 100

person-years (95% CI; 1.6-3.5). The difference observed was statistically significant (P=0.02) indicating that TB occurrence during HAART carried poor prognosis.

Table 3: Characteristics of incident tuberculosis cases at Zewditu Memorial Hospital September 11, 2006 to October 10, 2010

Characteristics	CD4 <100 cells/µl	CD4 ≥ 100 cells/µl	Total
	Frequency (%)	Frequency (%)	Frequency (%)
Patient developed TB			
Yes	30 (16.3)	21 (5.8)	51 (9.3)
No	154 (83.7)	341 (94.2)	495 (90.7)
Sex			
Male	15 (45.5)	8 (44.4)	23(45.1)
Female	18 (54.5)	10 (56.6)	28 (54.9)
Type of TB for incident cases			
Smear positive pulmonary	2 (6.7)	-	2 (3.9)
Smear negative pulmonary	11 (36.7)	11 (52.4)	22 (43.1)
Extra-pulmonary and/or Disseminated	17 (56.6)	10 (47.6)	27 (53.0)
Treatment outcome for TB cases			
Cured or completed	22 (73.4)	18 (85.6)	40 (78.4)
Dead	6 (20.0)	1 (4.8)	7 (13.8)
Still on treatment	1 (3.3)	1 (4.8)	2 (3.9)
NA	1 (3.3)	1 (4.8)	2 (3.9)
Side effect for anti-tuberculosis			
Yes	1 (3.3)	-	1 (2.0)
No	29 (96.7)	21 (100)	50 (98)
IPT provided			
Yes	1 (2.8)	-	1 (2.0))
No	34 (98.2)	16 (100)	50 (98.0)
CPT provided			
Yes	37 (94.9)	12 (100)	49 (96.1)
No	2 (5.1)	-	2 (3.9)
Past History of TB			
Yes	4 (9.3)	2 (25.0)	6 (11.8)
No	39 (91.7)	6 (75.0)	45 (88.2)

IPT=Isoniazid preventive therapy; CPT= Cotrimoxazole preventive therapy

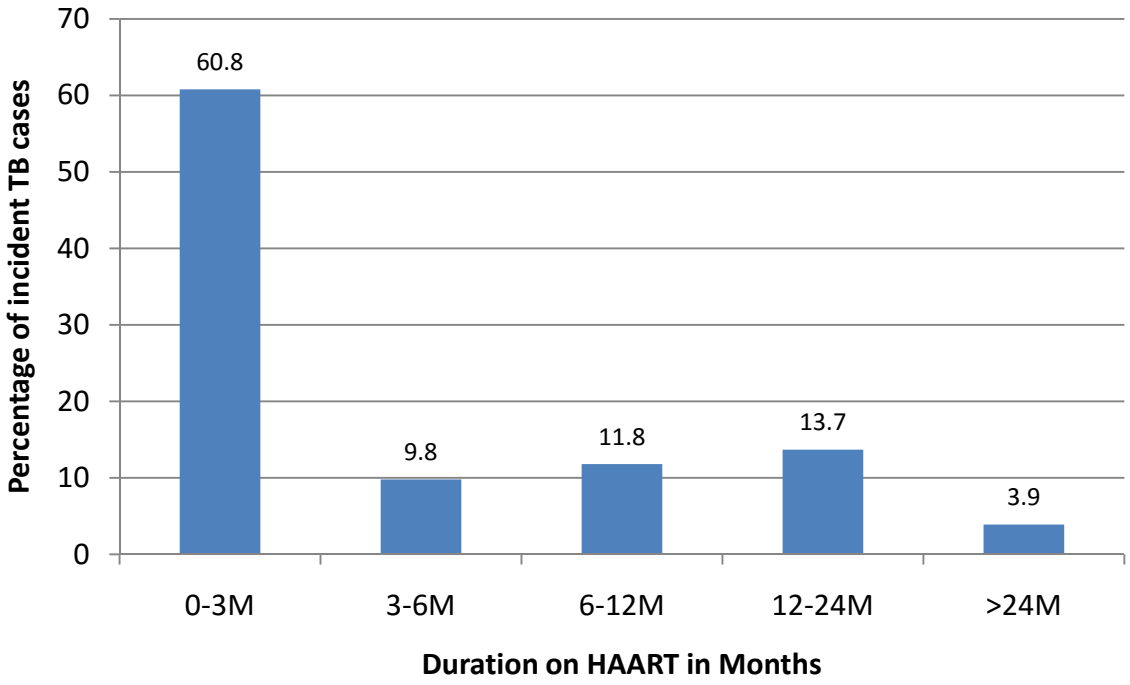

Figure 1: Time line for occurrence of tuberculosis in course of Highly Active Antiretroviral Therapy for patients at Zewditu Memorial Hospital September 11, 2006 to October 10, 2010

5.4. Tuberculosis Incidence Density Rate (IDR)

Tuberculosis incidence rates were calculated using person-years (PY) of follow-up as a denominator for the entire cohort and for groups classified based on socio-demographic and clinical characteristics (Table 4). The overall tuberculosis incidence density rate (IDR) in the cohort was 4.9 cases per 100PY [95% Confidence Interval (CI), 3.56-6.25]. Significant reduction of IDR was observed over the four-year period. The IDR was 43.8 cases per 100PY (95%CI; 26.6-61.0) for the first year, 4.7 cases per 100PY (95%CI; 1.2-8.3) for the second year, 0.4 cases per 100PY (95% CI; 0.4-1.2) for the third year and 0.2 cases per 100PY (95% CI; 0.16-0.5) for the fourth year (X^2 test for trend, P<0.001, Figure 2). There were no differences observed on major factors like baseline CD4 count, IPT use or clinical stage of the disease on the proportion

of remaining patients in the cohort from year to year. Therefore; changes in cohort composition could not explain the observed yearly difference in the incidence density rate.

Patients with nadir CD4 cell count of less than 100 cells/ µl had TB incidence of 9.8 cases per 100PY (95% CI; 6.3-13.3) compared to 2.9 cases of TB per 100PY (95% CI; 1.6-4.10) for patients with CD4 cell count of greater than or equal to 100 cells/ µl (P<0.001). The incidence of TB was the highest among patients with baseline CD4 cell count <50 cells/µl (10.2/100PY, 95% CI; 4.8-15.5). This was significantly higher when compared with the 4.1/100 PY (95% CI; 2.8-5.4) TB incidence among patients with CD4 count ≥50 cells/µl (P=0.01). The TB incidence of 5.5 cases per 100PY (95% CI; 3.8-7.2) observed for patients with symptomatic clinical disease at the start of HAART (WHO clinical stages 3 or 4] was significantly higher from the 3.4 cases per 100PY (95% CI; 1.3-5.5) incidence among WHO stage 1or 2 patients at baseline (P=0.01).

In patients who did not receive IPT, the incidence of TB was 5.3 cases per 100 PY (95% CI; 3.8-6.8) as compared to one case of TB per 100PY (95% CI; 0.97-3.0) among those who received IPT (P=0.02) indicating protective effect of IPT against TB. However, the incidence estimate of TB for patients who received IPT was variable as indicated by interval estimate due to small number of subjects in this category. No difference in the IDR was observed for socio-demographic factors (sex, age, educational status) and for clinical characteristics like past history of TB, baseline hemoglobin and use of cotrimoxazole preventive therapy (Table 4).

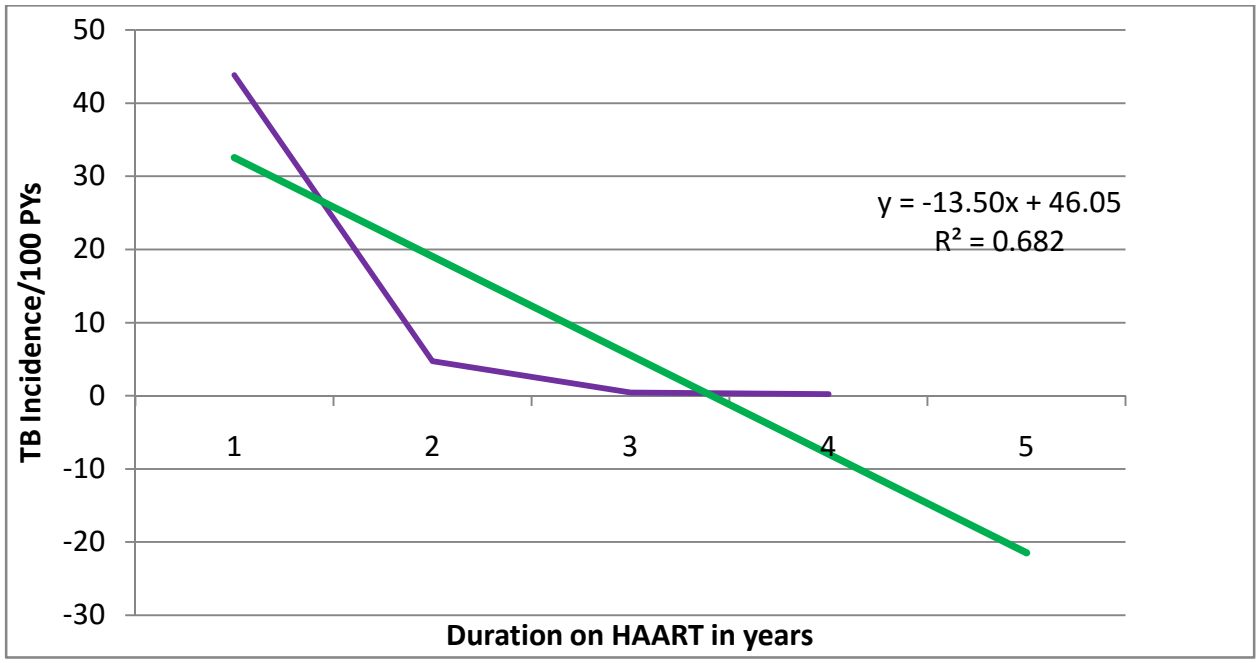

Figure 2: The decreasing trend of incidence of tuberculosis over the course of antiretroviral treatment

Table 4: Tuberculosis incidence density rate stratified by baseline socio-demographic and clinical characteristics of patients receiving HAART at Zewditu Memorial Hospital September 11, 2006 to October 10, 2010

Characteristics	No.of patients	Person-years	No.with TB	TB IDR (95%CI)	p-value [a]
Total patients	546	1039.35	51	4.91 (3.56-6.25)	
Age (years)					
15-29	106	162.49	11	6.77 (2.77-10.77)	
30-44	348	692.31	35	5.05 (3.38-11.68)	
≥45	92	184.55	5	2.71 (0.33-3.36	0.37
Sex					
Male	225	416.86	23	5.52 (3.26-7.77)	0.55
Female	321	622.42	28	4.50 (2.83-6.16)	
Occupation					
Employed	189	366.63	13	3.55 (1.62-5.47)	0.18
Unemployed	245	439.86	26	5.91 (3.64-8.18)	
Educational status					
Illiterate	62	123.57	4	3.24 (0.06-6.42)	0.39
Literate	477	903.98	47	5.20 (3.71-6.68)	
Past History of TB					
Yes	94	207.41	6	2.89 (0.58-5.1)	0.33
No	445	824.14	45	5.46 (3.86-7.05)	
Baseline CD4 cell count (cells/µl)					
<100	184	305.39	30	9.82 (6.31-13.34)	<0.001
≥100	362	733.84	21	2.86 (1.64-4.08)	
Baseline CD4 cell count (cells/µl)					
<50	89	137.4	14	10.19 (4.85-15.53)	0.01
≥50	457	901.9	37	4.10 (2.78-5.42)	
Baseline hemoglobin (gm/dl)					
<10	48	73.1	8	10.94 (3.36-18.53)	0.11
≥10	495	956.28	43	4.50 (3.15-5.84)	
WHO stage					
Stage 1 or 2	191	296.34	10	3.37 (1.28-5.47)	0.01
Stage 3 or 4	355	743.01	41	5.52 (3.83-7.21)	
IPT					
Yes	65	98.39	1	1.02 (-0.97-3.01)	0.02
No	481	940.96	50	5.31 (3.84-6.79)	
CPT					
Yes	511	959.67	49	5.12 (3.68-6.53)	0.76
No	35	79.69	2	2.51 -0.97-5.99)	

TB, Tuberculosis ; IDR, Incidence density rate (per 100 person-years); CI, confidence interval; IPT, Isoniazid preventive therapy; CPT, Cotrimoxazole preventive therapy, [a] obtained by X^2 test for the difference in TB IDR.

5.5. Risk Factor Analysis for the Occurrence of Tuberculosis

Cox proportional hazard analyses (bivariate and multivariate regression analyses) were used to determine risk factors for TB in patients receiving HAART. Factors found significantly associated with the occurrence of TB in the bivariate analyses were maintained for the multivariate analyses (Table 5). In addition, all factors with P-value of <0.3 in the bivariate analyses were entered in to multivariate analyses. Baseline CD4 count of <100 cells/ µl [adjusted hazard ratio; 2.8 (95%CI; 1.6-4.9, P<0.001] was a strong and independent risk factor for the occurrence of TB during HAART. Patients with symptomatic clinical disease (WHO stage 3 or 4) at entry had twice higher risk of acquiring TB in the course of HAART compared with WHO stage 1 or 2 patients at baseline [adjusted hazard ratio; 2.2 (95%CI;1.1-4.4, P=0.03)]. Patients who had not received isoniazid preventive therapy were at a greater risk of developing TB during HAART [unadjusted hazard ratio; 7.4 (95% CI; 1.02-53.57, P=0.048)] showing borderline significance. However, the risk estimate was not more accurate as evidenced by very wide interval estimation because only one patient developed TB from this category. In addition, the protective effect of IPT was not significant in the multivariate analyses [adjusted hazard ratio; 4.3 (95% CI; 0.6-32.2, P=0.15). Past history of TB was not associated with risk of TB in bivariate analysis but it was included in multivariate analysis a priori and there was a trend in multivariate analysis towards a past history of TB having a protective effect against TB during HAART [adjusted hazard ratio; 0.4 (95% CI; 0.2-0.9; P=0.04). Socio-demographic factors (age, sex, educational status, occupation) and baseline hemoglobin and use of CPT were not identified as risk factors for TB in patients receiving HAART (Table 5).

Table 5: Cox regression analysis of predictors of tuberculosis in HIV positive cohorts receiving HAART at Zewditu Memorial Hospital September 11, 2006 to October 10, 2010

Variable	No.with TB	No. with no TB	Bivariate analysis		Multivariate analysis	
			CHR, (95%CI)	P-value	AHR, (95% CI)	P-value [a]
Age (in years)						
15-29	11	95	2.0 (0.7-5.7)	0.2	1.4 (0.4-3.7)	0.6
30-44	35	313	1.9 (0.7-4.8)	0.2	1.1 (0.2-5.3)	0.7
≥45	5	87	1		1	
Sex						
Male	23	202	1.2 (0.7-2.1)	0.5		
Female	28	293	1			
Occupation						
Employed	13	176	1		1	
Unemployed	26	219	1.6 (0.8-3.1)	0.2	0.7 (0.3-1.3)	0.2
Educational Status						
Illiterate	4	58	0.7 (0.5-1.5)	0.4		
Literate	47	430	1			
Baseline Hemoglobin (gm/dl)						
<10	8	40	2.1 (1.0-4.4)	0.06	1.8 (0.7-4.4)	0.2
≥10	43	452	1		1	
Baseline CD4 cell count						
<100 cells/µl	30	154	3.3 (1.9-5.7)	<0.001	2.8 (1.6-4.9)	<0.001
≥100 cells/µl	21	341	1			
WHO stage						
Stage 1 or 2	10	181	1		1	
Stage 3 or 4	41	314	2.4 (1.2-4.8)	0.01	2.2 (1.1-4.4)	0.03
Past history of tuberculosis						
Yes	6	88	0.62 (0.26-1.46)	0.27	0.4 (0.2-0.9)	0.04
No	45	400	1		1	
IPT						
Yes	1	64	1		1	
No	50	431	7.40 (1.02-53.57)	0.048	4.3 (0.6-32.2)	0.15
CPT						
Yes	49	462	1			
No	2	33	0.59 (0.14-2.42)	0.46		0.76

HR=Hazard ratio, AHR= adjusted hazard ratio; CHR=Crude hazard ratio, CI= Confidence interval Note: All variables with a P-value of <0.3 in bivariate analyses were included in multivariate analyses.

The Kaplan-Meier estimates of TB-free survival proportion after initiation of HAART were made for the overall cohort and among patients stratified by factors like baseline CD4 cell count and WHO stage of HIV infection. The TB-free survival proportion for the entire cohort over the four-year follow-up period was 0.88 (Figure 3). Patients with baseline CD4 cell count of <100 cells/ µl had significantly lower probability of not developing TB than in those with ≥100 cells/ µl (80% versus 92%; P< 0.001 using the log rank test, figure 4). On further lowering down the CD4 cut off point, patients with CD4 count <50 cells/µl had significantly lower probability of not developing TB compared to those with CD4 count of ≥ 50 cells/µl (80% versus 88%, P=0.03). The effect of very low CD4 count (<50 cells/µl in this case) on the probability of TB occurrence did not show remarkable difference from CD4 count cut off value <100 cells/µl since there were only 89 patients with CD4 count of <50 cells/µl out of which only 14 developed TB during HAART. Likewise, patients with symptomatic clinical disease (WHO stage 3 or 4) had slightly lower probability of not developing TB compared with those with WHO stage 1 or 2 (87% versus 88%; P=0.03) over four years. However, the gap was wide in the first three-years of follow-up but converged as time of follow-up increased. For example, the chance for not acquiring TB at 40 months of follow-up for WHO stage 3 or 4 patients was 87% compared with 92% TB-free survival proportion for WHO stage 1 or 2 (figure 5) indicating that WHO stage of the disease at entry was not a strong predictor of TB occurrence for patients who stayed on HAART for long time. The four-year TB-free survival proportion was also determined for patients based on the use of IPT during HAART (figure 6). Patients who were not on IPT at any time in the course of treatment had lower four-year TB-free survival proportion as compared to those who received IPT (87% versus 97%; P=0.03).

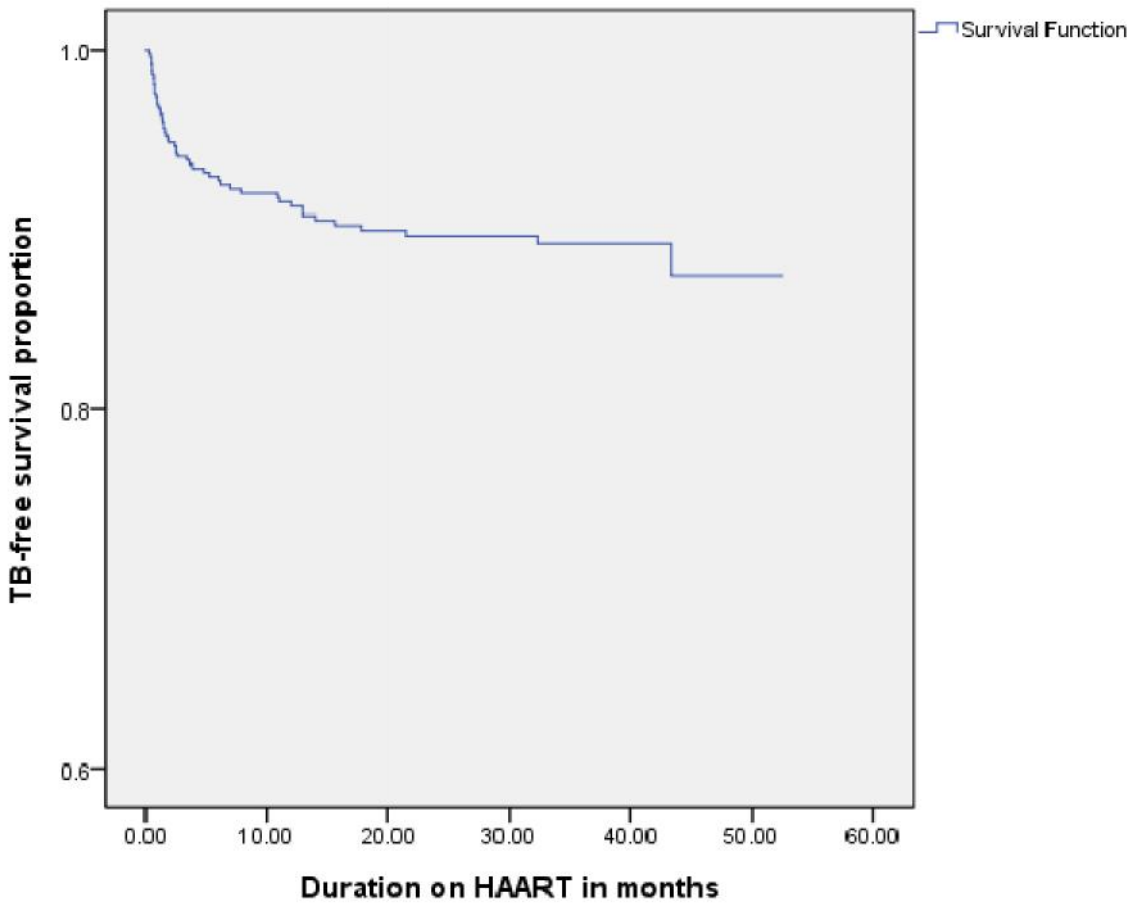

Figure 3: Kaplan-Meier plot of tuberculosis (TB)-free survival proportion for patients on Highly Active Antiretroviral Therapy at Zewditu Memorial Hospital September 11, 2006 to October 10, 2010

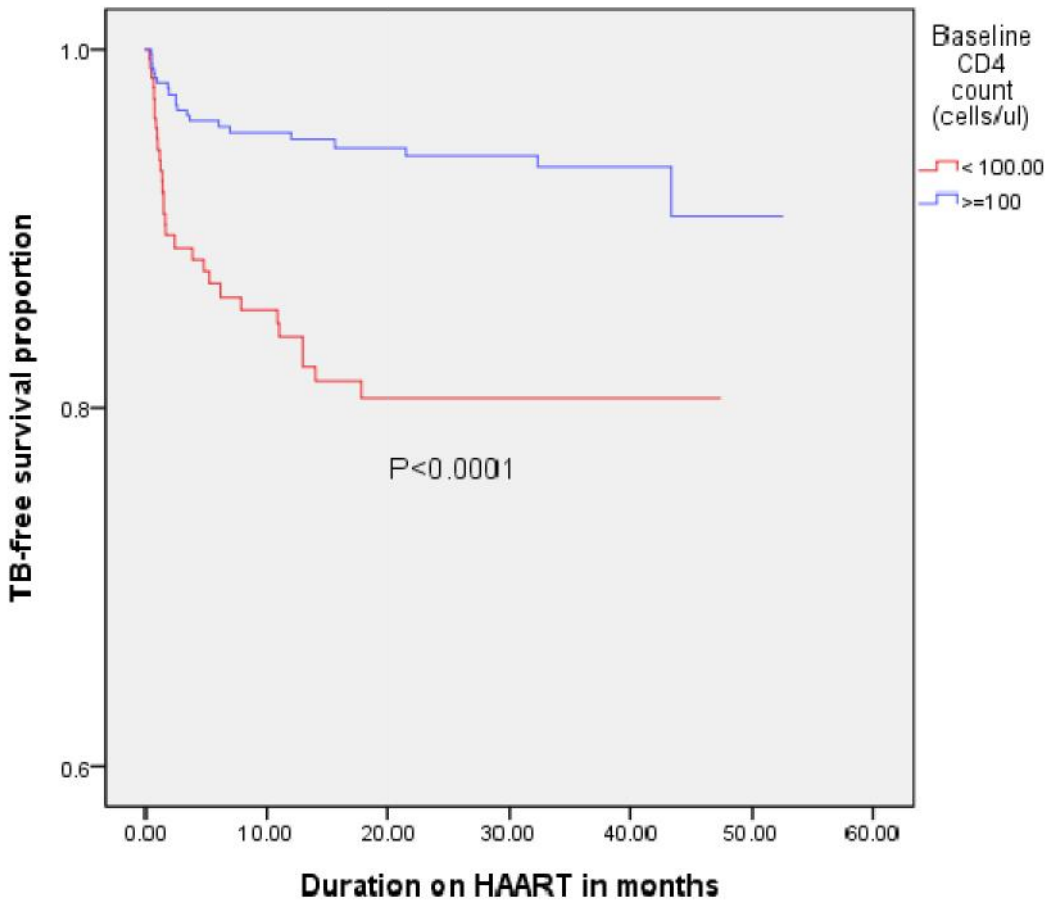

Figure 4: Kaplan-Meier plot of tuberculosis (TB)-free survival proportion based on baseline CD4 cell count (P-value from the Log rank test is given)

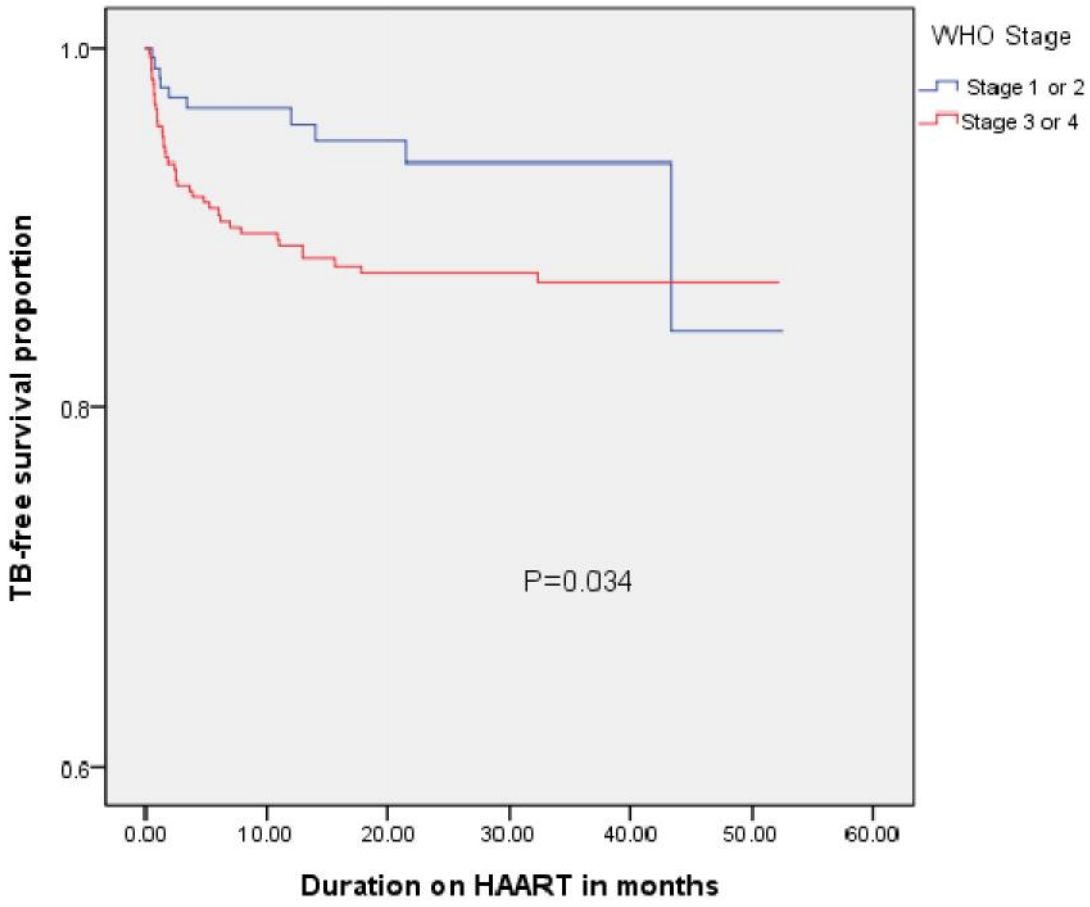

Figure 5: Kaplan-Meier plot of tuberculosis (TB)-free survival proportion based on clinical stage of the disease at entry (P-value from the log rank test given)

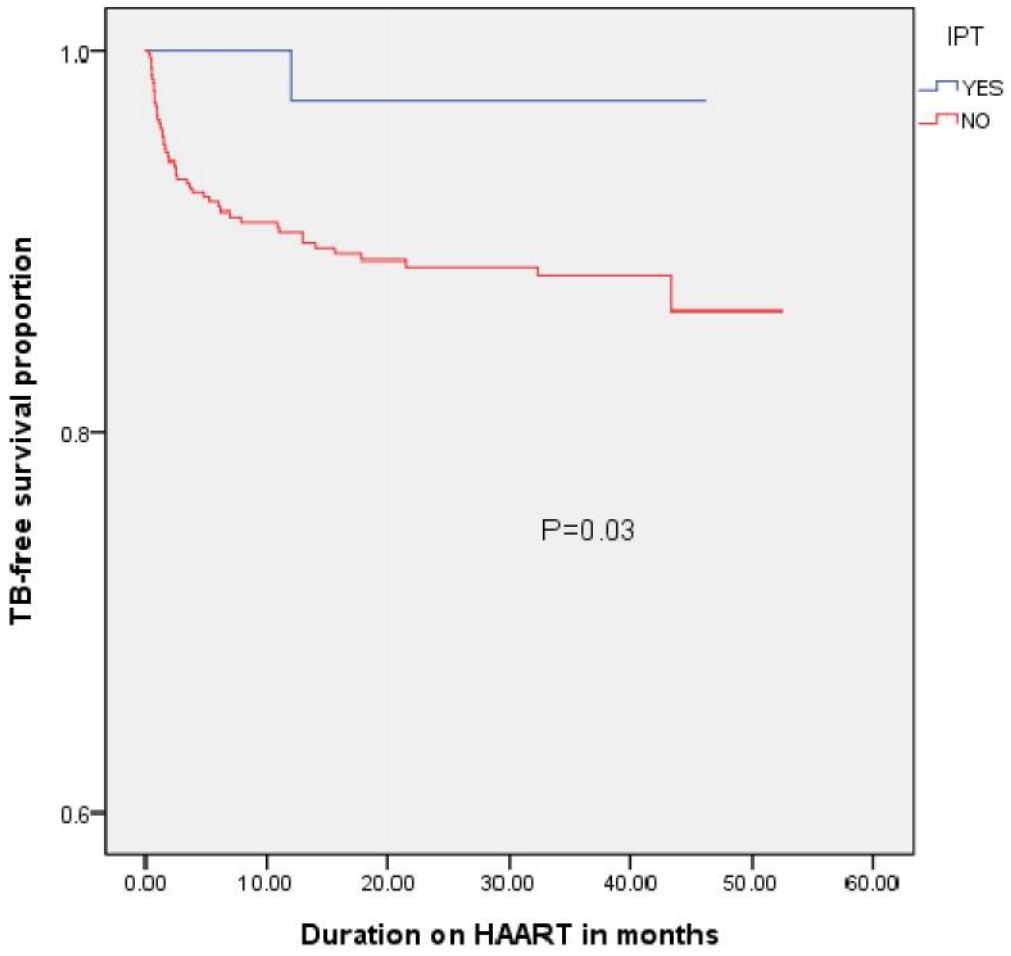

Figure 6: Kaplan-Meier plot of tuberculosis (TB)-free survival proportion based on the use of isoniazid preventive therapy (IPT)

6. DISCUSSION

Tuberculosis and the emerging multi-drug resistant TB epidemic pose major challenges both for the national HIV and TB control programs in resource-limited settings (32). HAART benefits the individual patients from the occurrence of repeated opportunistic infections and reduces the incidence of TB in treated cohorts by approximately 80% (14). However, unlike other opportunistic infections, tuberculosis remains the most frequent infection and a leading cause of death among patients receiving HAART particularly during the first months of treatment (33). This study confirmed that in Ethiopia tuberculosis is still a common HIV- related complication among patients receiving HAART with an incidence of 4.9 cases per 100PY (95% CI; 3.6-6.2).

The incidence showed a significantly decreasing trend over four-year follow-up period; decreasing from 43.8 cases per 100 PY in the first year to 0.2 cases per 100 PY in the fourth year. This time dependent change in TB incidence has been demonstrated in other similar long-term follow-up studies (15, 31, 34). However, the incidence of TB in the first year of HAART in this particular study was very high. This partly could be explained by the fact that nearly 80% of patients in this cohort had baseline CD4 count of <200 cells/ µl indicating advanced immunosupression, a major risk factor for TB. Among the new TB cases, 60.8% occurred in the first three months of HAART and 53% were extra-pulmonary and/or disseminated both of which may support Immune Reconstitution Inflammatory Syndrome (IRIS) as a factor to cause post-HAART tuberculosis. In developing countries where HIV and TB co-infections are very common, IRIS had been implicated for 11-47% of post-HAART tuberculosis (35). In addition, some clinical cases might have been left undiagnosed at HAART initiation due to lack of stringent TB screening practices or sensitivity of diagnostic techniques with persistent immunodeficiency or both. Nevertheless, a TB incidence of 0.2 cases per 100 PY even after the

fourth year on HAART indicated that HIV patients on treatment still were at higher risk for tuberculosis and occurrence of TB during HAART carried bad prognosis; mortality rate of 29.1 cases per 100PY as compared with 2.6 per 100 PY without TB (P=0.02).

Baseline CD4 cell count of <100 cells/ µl was a very strong and independent risk factor associated with a higher risk of TB in patients receiving HAART [adjusted hazard ratio; 2.8 (95% CI; 1.6-4.9), P<0.001]. In fact, the highest TB incidence (10.2/100PY) in this cohort analysis was observed among patients with baseline CD4 count <50 cells/µl. Lower baseline CD4 count before initiation of HAART has consistently been indicated as an independent risk factor for occurrence of TB during the course of HIV treatment in different settings (15, 16, 31, 34). One study in West Africa has indicated that baseline CD4 count has no any association with the occurrence of TB during HAART (17). This was because the study had profound limitations in design related to size of the study population, the number of TB cases, diagnostic criteria for TB and restricted cohort composition. In multivariate analysis in our study, WHO clinical stage 3 or 4 patients carried significant risk of developing TB during follow-up compared to stage 1 or 2 patients specially in the first 2-3 years of follow-up (table 5). Unlike baseline CD4 cell count, WHO clinical stage was not strong predictor of occurrence of TB as shown by the convergence of survival curve after prolonged duration of HAART (Figure 5).

In general, the findings in this study indicated that more advanced pre-treatment immunodeficiency (as indicated by very low baseline CD4 cell count and WHO clinical stage 3 or 4) retains the highest risk of TB during HAART which was consistent with results from studies involving untreated patients (10). This may reflect the fact that the greater the degree of

pre-HAART immunodeficiency, the more prolonged the period of treatment required to restore immune function. In addition, patients with advanced pre-treatment immunodeficiency are more likely to have suboptimal immunological recovery, which has been reported both in low-and high-income settings (26, 36-38). HIV treatment criteria in Ethiopia include only those with CD4 count of ≤200 cells/ µl, WHO stage 4 disease and WHO stage 3 disease with certain conditions (27). With similar argument, treatment based on this recommendation may limit the extent to which long-term TB-specific immune responses may be restored in many patients. This may restrict the potential benefit of HAART for the TB control. This was evidenced in this study by the fact that more than 80% of patients had baseline CD4 count of <200 cells/ µl and even after 4 years on HAART, the incidence of TB was still high at the level of 0.2/100PY (200/100,000).

Conflicting results have been reported for the association of past history of tuberculosis with the subsequent occurrence of TB in patients receiving HAART. The risk of tuberculosis after HAART was significantly higher in patients with history of TB than in those with no history of TB in one West African study (17). This was based on 129 adults enrolled in cohort out of which only 31 had previous history of TB and subjects were followed only for short duration. Studies with very large sample size and longer duration of follow-up have identified that past history of tuberculosis was not an independent risk factor for incidence of TB among patients receiving HAART (15, 39). In fact a 5-year cohort analysis in South Africa after a median duration of follow-up of 40 months demonstrated history of tuberculosis as protective factor for the occurrence of TB even though there was no significant statistical association (15). Similarly, in the bivariate analysis in this study, past history of TB was not associated with incidence of TB in the course of HAART. However; it showed some level of protection against TB in multivariate

analysis [adjusted hazard ratio; 0.4 (95% CI; (0.2-0.9), P=0.04). It is difficult to explain this association as complete restoration of immunity against TB in HIV infected patients will either not be possible or be prolonged after initial episode of TB (14, 31). It also was not possible to identify the incident TB cases whether these were due to re-infection or reactivation of latent TB. Nevertheless, it has been indicated that in countries with higher TB rates, most recurrences after appropriate treatment are probably due to re-infection rather than relapse and therefore, difference in the exposure could be mentioned as a confounding factor in this case (40).

The incidence of tuberculosis decreased from 6.7 per 100 PY for age group of 15-29 to 2.7 cases per 100 PY for patients with age ≥45 years. The trend was not statistically significant in the bivariate and multivariate Cox regression analysis indicating age was not risk factor for occurrence of TB among patients receiving HAART in this study. Similarly, lack of association between age and incidence of TB during antiretroviral treatment has been reported by other studies (31, 39). On the other hand, younger age has been described as risk factor for TB during HAART (15, 16). The later was difficult to explain as older age usually is associated with a higher risk of tuberculosis infection and disease. However, possibility of behavioral differences that affect exposure to HIV and subsequently to TB have been mentioned as an explanation for a decreasing trend of TB incidence in older age (15).

Male gender has been historically linked to an increased TB incidence (41). This may be attributable to illness behavior like symptom denial at time of presentation for ART initiation leading to higher rates of immune reconstitution TB. Male sex has also been associated with increased delay in diagnosis of TB (42). Likewise, significant association of male gender with

risk of tuberculosis among patients receiving HAART has been described in long term cohort studies (31, 39). Another study indicated male sex to be an important risk factor for the occurrence of TB only in the first three months of HIV treatment not then after (43) while others have indicated that male sex is not associated with an increased risk of TB in the course of HIV treatment (15, 16, 34). The incidence of TB among male patients on antiretroviral therapy in this cohort study was 5.5 cases per 100 PY compared with 4.5 cases per 100 PY for females (P=0.5), indicating absence of significant association in the difference of the incidence density rate between sex. A sub-analysis assessing risk factors of TB in the first three months alone also showed that male sex is not predictor of TB in patients receiving HAART.

Most cohort analyses dealing with establishment of TB incidence among HAART recipients universally reported that most TB episodes occur in the first 3-6 months. In this study, 61% of new TB cases were noted in the first three months after initiation of HAART and 70% occurred in the first 6 months. The overall incidence of TB on HAART was high (4.9 cases per 100 PY). The high TB incidence on HAART noted in this study and many others raises the issue of TB screening in all patients at HAART initiation and provision of isoniazid preventive therapy (IPT) to prevent incident cases (44). In Brazil and South Africa, in a much higher TB incidence setting for example, comparing TB incidence rates between cohorts receiving no HAART, HAART only or IPT and HAART showed that IPT can reduce the risk of TB beyond the reduction due to HAART and called for a wider use of IPT combined with HAART (16, 45).

In this cohort analysis, the incidence of TB among patients who have not received IPT at any time in the course of HAART was 5.3 cases per 100PY when compared with 1 case per 100 PY

among those who received IPT in addition to HAART (p=0.02). Bivariate Cox regression analysis showed borderline significance of the protective effect of IPT from occurrence of TB during HAART (Table 5). In addition, the Kaplan-Meier survival analysis indicated that patients on HAART who also received IPT, after four-years of follow-up have 10% higher TB-free survival proportion than those who received HAART only (Figure 6). However, the protective effect of IPT could not be established in multivariate Cox proportional hazard analysis [adjusted hazard ratio; 0.23 (95% CI; 0.03-1.7), P=0.15]. It should be noted that only 11.9% of patients in this cohort received IPT and analysis was made by an intention-to-treat, there by considering all patients who began IPT equally, regardless of their completion status. Despite the presence of enough evidence on protective effect of IPT in HIV patients, recommendation by national guidelines, the use of IPT in this cohort was very low. Challenges to exclude subclinical TB at baseline and the early high TB incidence during HAART demonstrated in this study and by others could delay IPT up to few months.

7. STRENGTHS AND LIMITATIONS OF THE STUDY

Unlike the previous cohort study done in Ethiopia (12), this study was able to describe the long-term impact (over four years) of HAART on TB incidence and the different risk factors associated with high TB incidence were elaborated. The analyses also allowed seeing the time-dependent changes in incidence of TB over four years. In addition, the combined effect of IPT and HAART on the incidence of TB was assessed in comparison with the provision of antiretroviral treatment alone. The study has also tried to explore the treatment outcome of the occurrence of TB among patients receiving HAART which was not possible in previous studies.

This study has some limitations. This was a retrospective cohort analysis where data were abstracted from medical records and may suffer from missing information even though the data abstraction process was closely monitored and extensive quality assurance measures were employed. For example, it was not possible to compare the immune response after treatment in both TB and non-TB cases after certain duration of HAART since CD4 cell count was not being done regularly for most subjects. Viral load indicated by many studies as predictor of prognosis was not included in the analysis as it was not available for almost all patients. Most patients have CD4 cell count less than 200 cells/ µl and incidence of TB could not be seen in patients with higher CD4 cell count, 350 cells/ µl or above for example. Almost all of the new TB cases were presumptive cases based on sputum smear microscopy, chest X-ray or clinical diagnosis. The absence of microbiological confirmation of TB cases which may lead in to possible under-or over-diagnosis of TB, therefore, was another limitation. However, this is the current practice in many sub-Saharan Africa settings. Even though the sample size was enough, the presented data was only from one center, possibly affecting generalizability of the findings.

8. CONCLUSION

This long-term cohort analysis with a median follow-up duration of 23 months estimated the incidence of tuberculosis in patients receiving HAART as 4.91/100 person-years. The first 3-6 months on HAART place the patients at high risk of developing tuberculosis. The incidence of tuberculosis in HIV patients receiving HAART showed a significant and time dependent decreasing trend over the years. This indicates that long-term HAART confers a greater reduction in risk of tuberculosis and as such HAART may contribute more to the TB control program in this setting. However, the study has also shown that the incidence of TB after four years on HAART was still higher calling for attention to other measures to effectively control TB in HIV patients. Profound baseline immunodeficiency as shown by low CD4 count (<100 cells/µl) and WHO clinical stage 3 or 4 were independent risk factors for tuberculosis in the course of antiretroviral treatment but none of socio-demographic factors were associated with high risk of TB in patients on HAART. Isoniazid preventive therapy (IPT) increased the TB-free survival proportion by 10% in patients receiving HAART after four years on HAART, though only 11.9% of patients were given IPT in this cohort. Tuberculosis treatment outcome analysis of incident cases has demonstrated that tuberculosis during HAART carries bad prognosis even though death could not be attributed to TB due to lack of information on causes of death in this study.

9. RECOMMENDATIONS

- The incidence of TB among HIV patients on treatment was very high and requires consistent implementation of comprehensive TB control measures including stringent screening for TB before initiation of HAART
- HIV patients should be enrolled to treatment at higher CD4 count as recommended in national guideline to reduce the incidence of TB (currently one-third of patients are enrolled in to treatment at CD4 count <100 cells/µl)
- The occurrence of one-third of TB cases with in the first month of HAART underscores the importance of improved TB screening among HIV patients before initiation of HAART including introduction of better diagnostic techniques
- The risk of TB remains higher even after long term treatment and this warrants better utilization of adjunct strategies like IPT
- Prospective cohort study is recommended to demonstrate long-term restoration of immune cell functions and to explore other strategies to reduce the TB rates further during HAART.

10. REFERENCES

1. MacDougall DS: TB & HIV: the deadly intersection. J Int As soc Physicians AIDS Care. 1999; **5**(5)**:**20-27.
2. WHO, "Global tuberculosis control-epidemiology, strategy, financing," WHO Report, Geneva, Switzerland, 2010. Available from: http://whqlibdoc.who.int/publications/2010/9789241564069_eng.pdf
3. Dakito DG, Yassin M A, Chekol LT, Kabeto LE, and Lindtjørn B. The rate of TB-HIV co-infection depends on the prevalence of HIV infection in a community.BMC Public Health. 2008; **8**:266.
4. Bruchfeld J, Aderaye G, Palme IB, Bjorvatn B, Britton S, Feleke Y, et al. Evaluation of outpatients with suspected pulmonary tuberculosis in a high HIV prevalence setting in Ethiopia: clinical, diagnostic and epidemiological characteristics. Scand J Infect Dis. 2002; **34**(5):331–7.
5. Yasin M, Takele L, Gebresenbet S, Girma E, Lera M , Lendebo E, Cuevas L. HIV and Tuberculosis Co-infection in the Southern Region of Ethiopia: A Prospective Epidemiological Study. Scand J Infect Dis. 2004; **36**:670-673.
6. Kassu A, Mengistu G, Ayele B, Diro E, Mekonnen F, Ketema D et al. Coinfection and clinical manifestations of tuberculosis in human immunodeficiency virus-infected and –uninfected adults at a teaching hospital, northwest Ethiopia. J Microbiol Immunol Infect. 2007 Apr; **40**(2):116-122.
7. Bekele A, Fleming K, Habtamu Z, Fogarty A. The association of TB with HIV infection in Oromia Regional state, Ethiopia in 2006/7. Ethiop.J.Health Dev. 2009; **23**(1):63-67
8. World Health Organization. Strategic Framework to Decrease the Burden of TB/HIV. [Document WHO/CDS/TB/2002.296, WHO/HIV_AIDS/2002.2. 2002.] Geneva: World Health Organization, 2004.
9. Lawn SD, Kranzer K, and Wood R. Antiretroviral therapy for control of the HIV-associated tuberculosis epidemic in resource-limited settings. Clin Chest Med. 2009; **30**(4):685-99.

10. Williams BG, Dye C. Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. Science. 2003; 301:1535-1537.
11. Lawn SD, Myer L, Edwards D, Bekker LG, Wood R. Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. AIDS. 2009; **23**(13):1717-25.
12. Jerene D, Lindtjørn B, and Næss A. Antiretroviral therapy at a district hospital in Ethiopia prevents death and tuberculosis in a cohort of HIV patients. AIDS Research and Therapy. 2006; **3:**10
13. Badri M, Wilson D, Wood R. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. Lancet 2002; **359**:2059-2064.
14. Lawn SD, Bekker LG, Wood R. How effectively does HAART restore immune responses to *Mycobacterium tuberculosis*? Implications for tuberculosis control. AIDS. 2005; **19**:1113-1124.
15. Stephen D L, Motasim B, and Robin W. Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort. AIDS. 2005; **18**(19): 2109-2116.
16. Jonathan E G, Valeria S, Solange C, Antonio G P, Laurence H M, Bonnie S K, et al. The impact of antiretroviral therapy and isoniazid preventive therapy on tuberculosis incidence in HIV-infected patients in Rio de Janeiro, Brazil. AIDS: 2007; **11** (21) 1441-1448.
17. Seyler C, Toure S, Messou E, Bonard D, Gabillard D, Anglaret X. Risk factors for active tuberculosis after antiretroviral treatment initiation in Abidjan. Am J Respir Crit Care Med. 2005; **172**:123-127.
18. Zumla A, Mwaba P, Huggett J, Kapata N, Chanda D, and J. Grange, "Reflections on the white plague," The Lancet Infectious Diseases. 2009. **9**(3):197–202.
19. Federal Democratic Republic of Ethiopia, Federal HIV/AIDS Prevention and Control Office. Report on progress towards implementation of the UN Declaration of Commitment on HIV/AIDS, 2010. Available from: (http://data.unaids.org/pub/Report/2010/ethiopia_2010_country_progress_report_en.pdf)

20. Lawn, S and Tutu, D. The challenges of HIV-associated tuberculosis epidemic in Sub-saharan Africa: will antiretroviral therapy help? Quarterly update on epidemiology from the South African Center for epidemiologic modeling and analysis. 2010; 2.
21. De Cock KM, Chaisson RE. Will DOTS do it? A reappraisal of tuberculosis control in countries with high rates of HIV infection. Int J Tuberc Lung Dis. 1999;**3**(6):457-65
22. World Health Organization.The Stop TB Strategy 2006.Available from: http://www.who.int/tb/strategy/en/
23. Federal HIV/AIDS prevention and control Office (FHAPCO). ART scale-up in Ethiopia: Successes and challenges. January 2009.
24. Wood R, Maartens G, Lombard CJ. Risk factors for developing tuberculosis in HIV-1-infected adults from communities with a low or very high incidence of tuberculosis. J Acquir Immune Defic Syndr. 2000; **23**:75-80.
25. Lange CG, Lederman MM, Medvik K, Asaad R, Wild M, Kalayjian R, *et al*. Nadir CD4+ T-cell count and numbers of CD28+ CD4+ T-cells predict functional responses to immunizations in chronic HIV-1 infection. AIDS. 2003; **17**:2015-2023.
26. Hsieh SM, Hung CC, Pan SC, Wang JT, Tsai HC, Chen MY, *et al*. Restoration of cellular immunity against tuberculosis in patients co-infected with HIV-1 and tuberculosis with effective antiretroviral therapy: assessment by determination of CD69 expression on T cells after tuberculin stimulation. J Acquir Immune Defic Syndr. 2000; **25**:212-220.
27. National Antiretroviral treatment guidelines in Ethiopia 2008. Available from: http://www.etharc.org/resources/download/view.download/40/235
28. Harries AD, Chimzizi RB, Nyirenda TE, van Gorkom J, Salaniponi FM. Preventing recurrent tuberculosis in high HIV-prevalent areas in sub-Saharan Africa: what are the options for tuberculosis control programmes? Int J Tuberc Lung Dis. 2003;**7**: 616–622
29. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet. 2001; **358**:1687–1693.

30. Corbett EL, Mallory KF, Grant AD, Churchyard GJ, De Cock KM. HIV-1 infection and the risk of tuberculosis after rifampicin treatment. Lancet. 2001; **357**:957–958.
31. Hermans SM, Kiragga AN, Schaefer P, Kambugu A, Hoepelman AIM, Manabe YC. Incident tuberculosis during antiretroviral therapy contributes to suboptimal immune reconstitution in a large urban HIV clinic in Sub-Saharan Africa. PLoS ONE. 2010 May; **5**(5): e10527.
32. Havlir DV, Getahun H, Sanne I, Nunn P. Opportunities and challenges for HIV care in overlapping HIV and TB epidemics. JAMA 2008; **300**: 423-30.
33. Lawn S, Harries A, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. AIDS 2008;**22:**1897-908.
34. Jean-Francois E, Assane D, Pierre DB, Akoi K,Ndeye Fatou N, Ibraham N,*et al.* Short and long-term incidence of Tuberculosis and CD4 cell count dynamics on HAART in Senegal. AIDS 2009; **3:** 63-70.
35. Haar CH, Cobelens FG, Kalisvaart NA, van Gersen PJ, van der Have JJ. HIV-related mortality among tuberculosis patients in The Netherlands, 1993-2001. Int J Tuberc Lung Dis 2007; 11:1038-41.
36. Nash D, Katyal M, Brinkhof MW, Keiser O, May M, *et al.* Long term immunologic response to antiretroviral therapy in low-income countries: a collaborative analysis of prospective studies. AIDS 2008; **22**:2291-2302.
37. Moore DMM, Harris RP, Lima VP,Hogg BP, May MP, *et al.* Effect of baseline CD4 cell counts on the clinical significance of short term immunologic response to antiretroviral therapy in individuals with virologic suppression. J Acquir Immune Defic Syndr 2009;**52:** 357-363.
38. Chi BHM, Giganti MM, Mulenga PLM, Limbada MM, Reid SEMM, *et al.*CD4+ response and subsequent risk of death among patients on antiretroviral therapy in Lusaka, Zambia. J Acquir Immune Defic Syndr 2009; 52: 121-131.
39. Rajasekaran S, Raja K, Jeyaseelan L, Vijila S, Priya K, *et al.* Post-HAART tuberculosis in adults and adolescents with HIV in India: Incidence, clinical and immunological profile. Indian J Tuberc 2009;**56:** 69-76.

40. van Rie A, Warren R, Richardson M, Victor TC, Gie RP, Enarson DA, *et al*. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. *N Engl J Med* 1999; **341**(16):1174-9.

41. Jarrin I, Geskus R, Bhaskaran K, Prins M, Perez-Hoyos S, Muga R, *et al*. Gender differences in HIV progression to AIDS and death in industrialized countries: slower progression following HIV seroconversion in women. Am J Epidemiol 2008, **168**: 532-540.

42. Meintjes G, Schoeman H, Morroni C, Wilson D, Maartens G. Patients and provider delay in tuberculosis suspects from communities with high HIV prevalence in South Africa: a cross-sectional study. BMC Infect Dis 2008; **8**:72-76.

43. Houlihan CF, Mutaverdzi PC, Lessells RJ, Cooke GS, Tanser FC,Newell M. The tuberculosis challenge in rural South Africa HIV programme. BMC Infect Dis 2010; **10**: 23.

44. Apers L, Lynen L, Worodria W, Colebunders R. Review editorial: Prevention of tuberculosis in resource poor countries with increasing access to highly active antiretroviral treatment. Trep Med Int Health 2005; **10**: 1209-14.

45. Golub JE, Pronk P, Lerato M. Isoniazid preventive therapy, HAART and Tuberculosis risk in HIV infected adults in South Africa: a prospective cohort. AIDS 2009; **23**: 631-6.

11. ANNEXES

Annex 1. Data collection tool

Incidence and risk factors of tuberculosis in patients receiving ART at Zewditu Memorial Hospital from September 11, 2006 to October 10, 2010, data collection tool

Code No._____

Part I: Sociodemographic characteristics

S.No	Questions	Categories
101	Sex	Male ---------1 Female ------2
102	Age in years [enter number]	------Years (age in completed years)
103	Address	Urban ----------1 Rural ----------2 Not recorded----------3
104	Marital status	Never married/single -----1 Married ----------2 Separated/divorced--------3 Widowed ----- ----------4 Others (specify) ----------
105	Religion	Orthodox ----------1 Muslim ----------2 Protestant ----------3 Catholic ----------4 Others (specify) ----------
106	Educational status	No education ----------1 Primary ----------2 Secondary ----------3 Tertiary ----------4 Not recorded ----------5
107	Occupation	Employed----------1 Unemployed--------2 Not recorded--------3

Part II: Past Tuberculosis diagnosis and treatment history

201	Does the patient have past TB treatment history?	Yes----------1(proceed with202) No----------2 (go to question 301) Not recorded-----3 (go to question 301)
202	Date treatment started	DD/MM/YY _____(E.C.)
203	Date treatment completed	DD/MM/YY_____(E.C.)
204	Type of Tuberculosis	Smear positive pulmonary ----------1 Smear negative pulmonary----------2 Extra-pulmonary ----------3 Other ----------4 Not recorded ----------5
205	Treatment outcome	Cured ----------1 Defaulted ----------2 Treatment failure-----3 Transferred ----------4 Not recorded----------5

Part III: HIV testing, treatment and follow-up

301	Date HIV status was known	DD/MM/YY_____(E.C.)
302	Disclosure	Yes----------1 No----------2 Not recorded--------3
303	If Yes, to whom?	Family members----------1 Friends ----------2 Relatives----------3 Others ----------4 Not recorded----------5
304	Date ART started	DD/MM/YY_____(E.C.)
305	Regimen at the start	D4T/3TC/NVP----------1 ZDV/3TC/NVP----------2 D4T/3TC/EFV----------3 ZDV/3TC/EFV----------4 Other (specify)_____
306	Substitution of Regimen	Yes----------1 No----------2
307	If Yes, date substitution was made	DD/MM/YY_____(E.C.)
308	If Yes on 306, reason for substitution	Toxicity/side effects----------1 Pregnancy/risk of pregnancy ---------2 Due to new TB----------3 New drug available ----------4 Drug out of stock ----------5 Others (specify)_____
309	Was regimen switched to 2nd line?	Yes----------1 No----------2
310	If Yes, when was switching done?	DD/MM/YY_____(E.C.)
311	CD4 count at the start of ART	_____Cells/µl
312	Subsequent CD4 counts	At 6 months _____Cells/ µl At 12 months _____cells/ µl At 18 months _____cells/ µl At 24 Months _____Cells/ µl At 30 months _____cells/ µl At 36 Months _____cells/ µl

313	WHO clinical stage at initiation of ART	Stage 1 or 2----------1 Stage 3 or 4----------2 Not recorded----------3
314	WHO clinical stage at the last follow-up	Stage 1 or 2----------1 Stage 3 or 4----------2 Not recorded----------3
315	Viral load at the start of ART	_____copies /ml
316	Viral load at the end of follow-up	_____copies/ml
317	Weight at the start of ART	_____Kg
318	Weight at the last follow-up	_____Kg
319	Total lymphocyte count at the start of ART	_____cells/ml
320	Total lymphocyte count at end of follow-up	_____cells/ml
321	Hemoglobin at the start of ART	_____mg/dl
322	Hemoglobin at the end of follow-up	_____mg/dl
323	Functional status of Patient at the start of ART	Ambulatory----------1 Working ----------2 Bedridden----------3 Not recorded---------4
324	Date of last follow-up	DD/MM/YY_____(E.C.)
325	Date of death for those died	DD/MM/YY_____(E.C.)
326	Date of lost to follow-up or transferred outs	DD/MM/YY_____(E.C.)

Part IV: Tuberculosis screening information

401	Did Patient develop TB during follow-up?	Yes----------1(continue with 402---) No----------2 (proceed to 407---)
402	When was TB diagnosis made?	DD/MM/YY_____(E.C.)
403	Type of TB diagnosed	Smear positive pulmonary TB----------1 Smear negative pulmonary TB----------2 Extra-pulmonary TB----------3 Others specify _____ Not recorded----------4
404	Treatment outcome of Tuberculosis	Cured ----------1 Defaulted ----------2 Dead----------3 Treatment failure-----4 Transferred ----------5 Not recorded----------6
405	Did patient develop side effects to TB treatment?	Yes----------1 No----------2
406	Did patient develop side effects for ART?	Yes ----------1 No----------2
407	Has patient taken/is taking IPT?	Yes----------1 No----------2
408	If Yes, Start date for IPT	DD/MM/YY_____(E.C.)
409	If Yes on 405, End date for IPT	DD/MM/YY_____(E.C.)
410	Has patient taken/is taking CPT	Yes----------1 No----------2
411	If Yes, date of Start on CPT	DD/MM/YY_____(E.C.)
412	If Yes, end date for CPT	DD/MM/YY_____(E.C.)

Annex 2. Participant Information Sheet and Consent Form

Description of the study

Title of the study: Incidence and risk factors of tuberculosis among patients receiving HAART in Addis Ababa, Ethiopia.

Objective of the study: To assess the incidence and risk factors for tuberculosis in patients receiving HAART.

Introduction

Highly active antiretroviral therapy (HAART) reduces the incidence of TB by many folds by increasing CD4 count and through restoration of TB-specific immune system. Even though the incidence of TB is reduced significantly during treatment, the risk remains substantially higher as compared to HIV negative subjects. The long term effect of HAART on the incidence of TB is affected by multiple factors such as early initiation of treatment and optimal use of ART. In Ethiopia, patients are enrolled to treatment lately and there is little or no information on the long term impact of HAART on TB incidence. Factors associated with higher risk of TB during HAART are also not well characterized. Understanding the factors associated with occurrence TB during HAART will be helpful for better management of patients and provides additional information to formulate national TB/HIV management guidelines. Therefore; this study is aimed at determination of the incidence and risk factors of TB among patients receiving HAART. The proposal for the study has been approved by Institution Review Board (IRB) of Gondar University and Addis Continental Institute of Public Health. Information which is necessary for the study will be taken from your chart. Since the study will be conducted by taking appropriate information from your medical chart, it will not inflict any harm on you and the information will be taken only when you give permission, participation is totally voluntary. You will not face any problem if you don't allow the information to be taken from your chart and there will also be no negative consequences on your programs. Similarly, you will not get any incentive for agreeing to participate in the study. Your name or any other identifying information will not be recorded on the questionnaire and all information taken from the chart will be kept strictly confidential and in a safe place. The information retrieved will only be used for the study purpose.

12. DECLARATION

I, the undersigned declare that this thesis is my original work in partial fulfillment of the requirement for the degree of Master of Public Health. I also declare that it has never been presented in this or any other university and that all resources and materials used in the thesis have been duly acknowledged.

Student Name: _____

Signature: _____

Place of submission: _____

Date of submission: _____

This thesis has been submitted with my approval as a university advisor.

Advisor Name: _____

Signature: _____

Date of submission: _____